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(19)(CA) APPLICATION FOR CANADIAN PATENT (12)

(54) Preparation and Use of Gene Banks of Synthetic Human Antibodies ("Synthetic Human-Antibody Libraries")

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Abstract of the disclosure

Preparation and use of gene banks of synthetic human antibodies ("synthetic human-antibody libraries")

The invention relates to the preparation and use of gene banks of synthetic human antibodies (huAb) or parts of antibodies which contain the antigen-binding domain. Starting from a huAb framework in a suitable vector, the hypervariable regions of the antibody cDNA are formed by almost "randomly" combined oligonucleotides. Relatively conserved amino acids in the hypervariable regions have here been taken account of in the choice of appropriate nucleotides during the oligonucleotide synthesis and the ratio of the nucleotides used is likewise chosen such that a nonsense codon is to be expected at most in every 89th position. Expression of this synthetic huAb cDNA in microbial expression systems, e.g. in E. coli in the vector pFMT which is described below, thus makes a synthetic huAb library with a comprehensive repertoire for screening using selected antigens available in vitro.

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The invention relates to the preparation and use of gene banks of synthetic human antibodies (huAb) or parts of antibodies which contain the antigen-binding domain. Starting from a huAb framework in a suitable vector, the hypervariable regions of the antibody cDNA are formed by almost "randomly" combined oligonucleotides. Relatively conserved amino acids in the hypervariable regions have here been taken account of in the choice of appropriate nucleotides during the oligonucleotide synthesis and the ratio of the nucleotides used is likewise chosen such that a nonsense codon is to be expected at most in every 89th position. Expression of this synthetic huAb cDNA in microbial expression systems, e.g. in E. coli in the vector pFMT which is described below, thus makes a synthetic huAb library with a comprehensive repertoire for screening using selected antigens available _in vitro_.

The human or mammalian immune system comprises an estimated number of between 10^6 and 10^8 different antibodies. This number of antibodies seems to be sufficient to cause an immune reaction of the body both against all naturally occurring antigens and against artificial antigens. If it is furthermore taken into account that often different antibodies react with the same antigen, the repertoire of antibodies that are really different would rather be in the region from 10^6 to 10^7.

Up to now specific antibodies have always been obtained starting from an immunization with the particular antigen, for example injection of the antigen into the body or _in vitro_ incubation of spleen cells with this antigen. In the case of polyclonal antibodies, the immunoglobulins

can then be isolated from the serum and the specific antibodies can be isolated therefrom, e.g. by absorption methods. Monoclonal antibodies are isolated from the cell supernatants or from the cell lysate of spleen tumor cells (hybridoma cells) which have been fused with individual B lymphocytes and cloned. The abovementioned methods are unsuitable in particular for the preparation of specific human antibodies or human monoclonal antibodies.

The present invention therefore has the object of developing a generally usable method for generating specific human monoclonal antibodies (huMAbs) or parts of antibodies, which contain synthetic hypervariable domains.

It has now been found that by using almost randomly synthesized oligonucleotides coding for the three hypervariable regions of each variable part of heavy or light chains (called CDR1, 2 and 3, CDR meaning complementary determining region) synthetic human gene banks can be generated. The synthesized antibody DNA was then preferably ligated into an antibody expression vector especially constructed for this purpose, namely the vector pFMT, preferably after amplification using the polymerase chain reaction (PCR).

The oligonucleotides which are used for the synthesis of the variable domains of heavy and light chains are compiled in Tab. 1. Set A here contains fewer limitations than set B. The limitations below the synthesis of the hypervariable regions (see CDR regions in Tab. 4) being random were made in H3, H4, H6, L2, L3 and L5 in set A in order firstly to allow for positions in the sequence for certain conserved amino acids, secondly to reduce the number of stop codons, and thirdly to incorporate a new restriction site.

(a) In order to reduce the probability of the stop codon

occurring, only half the amount of the three other nucleotides was allowed for T at the first position of each codon and A was omitted at the third position of each codon, in each case. As a statistical average, only every 89th codon will thus be a stop codon.

(b) For the 2nd codon in the CDR1 region of the light chain, only those nucleotides were allowed which code for the amino acids V, A or G.

(c) Likewise, only those combinations coding for V, I or M were allowed for codon No. 10 in the CDR1 region of the light chain and for codon No. 4 in the CDR1 region of the heavy chains.

(d) In the CDR3 region of the light chain, only those nucleotides coding for the amino acid glutamine were allowed for codon No. 1.

(e) In the CDR2 region of the heavy chain, only those nucleotides coding for the amino acid tyrosine were allowed for codon No. 11.

(f) An A was advantageously incorporated at the third position of the last codon in the CDR2 region of the heavy chain in order to introduce a restriction site for MluI.

The random nature of these oligonucleotides was preferably limited even further in those positions where predominantly one or few amino acids occur (set B in Tab. 1, the limitations here are based on the tables by Kabat et al. (1987), Sequences of Proteins of Immunological Interest-U.S. Dept. of Health and Human Services, U.S. Government Printing Offices). A list of the corresponding nucleotides and brief notes on the codon combinations are compiled in Tab. 1 and in the notes for Tab. 1.

After ligation of equimolar amounts of the oligonucleotides H1 to H7 and L1 to L5, these are ligated into the pretreated expression vector pFMT. Preferably, a PCR step using the primers H1 and H8, or L1 and L6 should be carried out beforehand in order to amplify the amount of DNA. After producing suitable restriction sites at the ends of the antibody DNA using appropriate restriction enzymes, the DNA is ligated into the antibody expression vector pFMT in the same manner as above (see examples).

The expression pFMT makes possible the expression of antibody cDNA and the subsequent secretion of the expression products in bacteria (E.coli). The antibody operon of the plasmid contains the sequences of the variable parts of both the heavy and light chain of an antibody. Suitable leader sequences from the amino terminal part of a bacterial protein makes secretion of the antibody parts possible. The leader sequences are cleaved off by a bacterial enzyme during the secretion. During the secretion of the antibody cDNA products, the light and heavy chains of the antibody (with or without an adjacent constant domain) become associated. This results in the formation of an antibody or antibody fragment which, in either case, contains a functional antigen binding site. Similar constructs for individual antibodies have also been described by other authors (Better et al. (1988), Science 240, 1041, and Skerras & Plückthun (1988), Science 240, 1038).

In the synthetic human-antibody library formed by the expression in, for example, E. coli, the desired human antibodies or antibody parts are found by screening bacterial clones using the selected antigen. In a preferred embodiment, an additional sequence which codes for a marker peptide, for example a TAG sequence, is incorporated so that the expression products can be detected in a simple way using established monoclonal antibodies against the marker peptide (Wehland et al. (1984), EMBO J. 3, 1295).

The abovementioned exemplary formulations and the examples below shall be understood as illustrating but not restricting the invention.

The invention therefore relates to gene banks of synthetic huAb or antigen-binding parts thereof, obtained by means of (1) cDNA for the hypervariable regions generated on a random basis, where the random sequences are limited by clauses (a) to (e) set A or in accordance with Tab. 1, set B, (2) preferably a subsequent amplification step of these random sequences and (3) ligation of the said cDNA into a suitable expression vector, preferably pFMT, an additional coding sequence for a marker peptide being incorporated in a preferred embodiment.

The invention also relates to a process for the separation of the abovementioned gene banks, and the process and the use thereof for the isolation of clones which secrete specific antibodies or antigen-binding parts thereof.

Finally, the invention is explained in detail in the examples and contained in the patent claims.

Examples:

Example 1: Preparation of an antibody expression vector

The plasmid pKK233-2 (Amann and Brosius, (1985) Gene 40, and Straus and Gilbert (1985) Proc. Natl. Acad. Sci. 82, 2014) was chosen as base vector for the construction of the antibody expression vector (Fig. 1).

Before the incorporation of the antibody operon, the plasmid was cut with SalI and BamHI, the ends were filled in with Klenow polymerase and ligated. By doing so, the two restriction sites and the DNA between them were

deleted.

Additionally, the plasmid was cleaved with HindIII, the ends were filled in with Klenow polymerase and ligated using BamHI linkers. By this procedure, the HindIII restriction site was removed and a BamHI site inserted. The antibody DNA was inserted into this modified plasma. A simplified structure of the antibody operon coding for a dicistronic antibody mRNA is shown in Tab. 2. In order to make possible the secretion of the antibody, the leader sequence of the bacterial enzyme pectate lyase was used. The leader sequence of this enzyme has already been used for the expression and secretion of a chimeric murine/human antibody (Fab fragment, Better et al., loc. cit.), and of the variable region of a "humanized" antibody (Ward et al., loc. cit.; Huse et al., loc. cit.). DNA for the first leader sequence (P_1 upstream of the heavy chain), and the sequence for a second ribosome binding site (RBS) and a second leader sequence (P_2 upstream of the light chain) were synthesized from several oligonucleotides (Tab. 3).

Antibody cDNAs which code for the variable regions of the heavy and light chains of a human antibody (HuVhlys or HuVllys; Riechmann et al., (1988) J. Mol. Biol. 203, 825) were obtained from Dr. G. Winter (Cambridge, UK). The restriction sites HindIII (HuVhlys) and EcoRV (HuVllys) were introduced to make possible the insertion of the antibody cDNA into the expression vector. Further restriction sites for BanII (HuVhlys) and BstEII or KpnI (HuVllys) were introduced to exchange hypervariable regions en bloc. At the end of the HuVhlys cDNA sequence a stop signal was incorporated. A BanII site in the light chain was removed. These alterations were carried out by means of site directed mutagenesis in the bacteriophage M13mp18 (Zoller and Smith, Meth. Enzymol. 100, 468-500). The sequence of the completed antibody DNA is shown in Tab. 4.

For the insertion of the leader sequence P_1 (Tab. 3) the modified plasmid pKK233-2 was digested using the restriction enzymes NcoI and PstI, and P_1 was inserted in between these sites (pKK233-2-P_1). Further cloning steps, apart from the last step, were carried out using the plasmid pUC18. The reason is that the presence of individual parts of the antibody operon in the expression vector adversely influences the growth of the bacterial host.

Before the cloning in pUC18, its BamHI restriction site had to be removed. After digesting with BamHI, the single-stranded ends were filled in using the Klenow fragment and were religated. This modified plasmid was then digested using PstI and HindIII, and P_2 plus RBS was ligated in between the restriction sites (pUC18-P_2). During this process, the original HindIII restriction site of the plasmid disappears and a new HindIII restriction site is incorporated. pUC18-P_2 was then digested using pstI and HindIII, and the DNA of the heavy chain (PstI-HindIII insert from M13) was ligated into these two sites (pUC18-HP_2). This plasmid was then digested using EcoRV and BamHI, and the DNA of the light chain (EcoRV-BamHI insert from M13) was ligated in (pUC18-HP_2L).

The PstI-BamHI insert was then recloned in pUC18 after the restriction sites for HindIII, BanII and KpnI therein had previously been removed. The HindIII restriction site was removed as above for pKK233-2, the religation taking place without an insertion of BamHI linkers, however. Subsequently, the resulting plasmid was digested using SmaI and BanII, and, after filling in the protruding ends by means of T4 DNA polymerase, religated. The insertion of the PstI-BamHI restriction fragment results in pUC-HP_2L. In a preferred embodiment, a Tag sequence was additionally inserted in the BanII and HindIII restriction sites (Tab. 3). The Tag sequence encodes the recognition sequence Glu-Gly-Glu-Glu-Phe of the monoclonal antibody Yl 1/2 (Wehland et al., (1984), EMBO J. 3, 1295). Because

of this peptide marker the expression product of the resulting plasmid pUC-HTP$_2$L is readily detectable.

For the insertion of HP$_2$L or HTP$_2$L in the expression vector, the two plasmids were cut using PstI and BamHI, and the PstI-BamHI HP$_2$L insert from pUC-HP$_2$L or the HTP$_2$L insert from pUC-HTP$_2$L was ligated into the modified plasmid pKK233-2-P$_1$ into these two restriction sites. A diagrammatic representation of the completed expression vector pFMT is shown in Tab. 5.

Example 2: Synthesis of antibody DNA containing random sequences in hypervariable regions

The synthesized oligonucleotides for the synthesis of the variable parts of antibody DNA are compiled in Tab. 1. For the synthesis of the hypervariable regions almost random nucleotide sequences were used. Limitations on the random nature are illustrated in Tab. 1. Two different sets of oligonucleotides were synthesized. In set A the hypervariable regions are predominantly random apart from those few positions where almost exclusively certain amino acids occur. In set B, the random nature of the nucleotide sequences in those positions where predominantly one or few amino acids occur was additionally limited.

The oligonucleotides were purified by HPLC chromatography or polyacrylamide gel electrophoresis, and then 5'-phosphorylated.

Example 3: Ligation of the synthetic oligonucleotides

The oligonucleotides in Tab. 1 were ligated together stepwise on an antibody DNA template. For this purpose, large amounts (about 1 mg) of single-stranded M13mp=18 DNA containing the antibody DNA inserts were isolated. In order to separate the antibody DNA from the vector, the inserts were made double-stranded on the two ends using

two appropriate oligonucleotides and were digested using the enzymes PstI and HindIII (heavy chain) or using EcoRV and BamHI (light chain). The antibody DNA was then purified using agar gel electrophoresis.

On these DNA templates, first only three oligonucleotides were ligated: H1, pH2 and pH3 (heavy chain), and L1, pL2 and pL3 (light chain), H1 and L1 having been marked first with ^{32}P at their 5' end ("p" designates 5'-phosphorylated). Amounts of 100pmol of each oligonucleotide were used. The hydridized oligonucleotides were purified on 2% agarose gels and analyzed on a sequencing gel. The amount was determined by a radioactivity measurement. Equimolar amounts of pH4 and pH5 (heavy chain), and pL4 and pL5 (light chain) were then ligated onto the already ligated oligonucleotides on each particular template. These DNAs were then purified as in the preceding step and the procedure was repeated up to the purification step, using equimolar amounts of pH6 and pH7. Finally, the ligated oligonucleotides were purified by means of a denaturing polyacrylamide gel and preferably amplified using the polymerase chain reaction (PCR). Alternatively or in order to avoid losses caused by the last purification step, the oligonucleotides were amplified using PCR directly after the last ligation step. The primers H1 and H8 (heavy chain), and L1 and L6 (light chain) were used under standard conditions for the PCR. Amplified template DNA was digested selectively using KpnI (light chain) or using AluI (heavy chain). Where appropriate, a second amplification step using the PCR was subsequently carried out.

Example 4: Insertion of the antibody DNA into the expression plasmid

The synthesized antibody DNA was cut using the restriction enzymes PstI and BanIII (heavy chain), and BstEII and KpnI (light chain). The bands having the expected molecular weight were purified by agar gel

electrophoresis, precipitated using ethanol and then, in two steps (first the DNA of the light chain and then the DNA of the heavy chain), ligated into the pUC-HP_2L (see above) which had been cut and purified in the same way. The HP_2L insert was then ligated into the restriction sites PstI and BamHI of the plasmids pKK233-2-P_1 (see Example 1). An analogous way was used for the HTP_2L fragment. The antibody library is therefore established in the antibody expression plasmid (Tab. 6). The reason for intermediate cloning in pUC is that the presence of individual parts of the antibody operon in the expression vector has an adverse influence on the growth of the bacterial host (see above also).

Example 5: Expression and screening of antibodies in E. coli

Competent E. coli are transfected with pFMT plasmids containing the inserted antibody-DNA library, grown on agarose plates and then incubated using nitrocellulose filters coated with the desired antigen. After removing non-specifically bound antibodies, the active clones are identified with a labeled antibody against the human immunoglobulins secreted from E. coli. In the preferred embodiment, the antibody YL 1/2 which is directed against the Tag sequence is used for this purpose.

Legend for Fig. 1:

Restriction map of the expression vector pKK233-2 (Amann and Brosius, loc. cit.).

Ptrc denotes hybrid tryptophan lac promoter

RBS denotes ribosome binding site

rrnB denotes ribosomal RNA B operon

5S denotes gene for 5S RNA

Before cloning antibody DNA in the expression vector, the following alterations were carried out:

1) The SalI and EcoRI restriction sites were removed together with the DNA between them.

2) The HindIII restriction site was converted to a BamHI restriction site.

TAB. 1

Oligonucleotids for the synthesis of a library of anti-body DNA (variable parts)

Set A

```
H1     5'CCAGGTCCAACTGCAGGAGAGCGGTCCAGGTCTTGTGAGA.CTAG3'

H2     5'CCAGACCCTGAGCCTGACCTGCACCGTG3'

H3     5'TGTCTGGCTTCACCTTCAGC|T1/2 TT| CTTT1/2TTTGGGTGCGCCAGCCACCTGGAC3'
                 C         C |C     CC| A CC    CC
                             |A     AG| G AA    AG
                             |G     G |3  GG    G

H4     5'GAGGTCTTGAGTGGA.TGGT|T1/2TT| TAT|T1/2TT|  T1/2TACGCGTGACAATGCTGGTAGAC3'
                             |C    CC|     |C    CC|  C    C
                             |A    AG|     |A    AG   A    A
                             |G    G |10   |G    G |5 G    G

H5     5' ACCAGCAAGAACCAGTTCAGCCTGCGTCTCAGCAGCGTGACAGC3'

H6     5'CGCCGACACCGCGGTCTACTACTGTGCGCGC|T1/2 TT TGGGGTCAGGGCT3'
                                          C     CC
                                          A     AG
                                         |G     G  10

H7     5'CCCTCGTCACAGTCTCCTCA3'

H8     5'CTGTGACGAGGCTGCCCTGACCCCA3'

L1     5'GCGCCAGCGTGGGTGACAGG3'

L2     5'GTGACCATCACCTGTT1/2TTGTT|T1/2TT| CTTT1/2TTTGGTAACAGCAGAAGCCAGGT3'
                     C    CC CC|C    CC| A CC    CC
                     A    AG GA|A    AG| G AA    AG
                     G    G   G|G    G |7  GG    G

L3     5'AAGGCTCCAAAGCTGCTGATCTAC|T1/2TT| GGTGTGCCAAGCCGTTTCAGCGGTAGCGGT3'
                                 |C    CC
                                 |A    AG|
                                 |G    G |7

L4     5'AGCGGTACGGACTTCACCTTCACCATCAGCAGCCTCCAGCCAGAGGAC3'

L5     5'ATCGCCACCTACTACTGCCAG|T1/2TT|TTCGGCCAAGGTAC3'
                              |C    CC|
                              |A    AG|
                              |G    G |5

L6     5'CCACCTTGGTACCTTGGCCGAA3'
```

Set B

H1, H2, H5, H7, H8 and L1, L4 and L6 are identical to those in set A.

```
B3   5'TGTCTGGCTTCACCTTCAGC AC10%T95% TT20%C TC20%G T5% TA20% T2% T5% T70%
               C         C  GA45%A5%   C10%  GG80%  A35% G80% C28%A75%G30%
                             G45%      A70%         G10%      A50%G20%
                                                              G20%
TGGGTGCGCCAGCCACCTGGAC3'

B4      5'GAGGTCTTGAGTGGATTGGT T14.5%TT AT90%C T14.5%TT T5% T10%T70% T14.5%TT
                               C28.5%CC  G10%  C28.5%CC C70%C80%G3C% C28.5%CC
                                A28.5%AG       A28.5%AG A20%A10%      A28.5%AG
                                G28.5%g        G28.5%G  G5%           G28.5%G

AAT A15%A20%C ACT AT16%A A70%C10%C TAT A80%C20%C CCC AC10%C T40%TC C5% T5% G
GG  G85%G80%  GA   C80%  G30%A70%A     G20%A60%  GAA  A40%A C20%    A90%A90%
               G   A4%      G20%          G20%        G50%  G40%    G5% G5%

A20%A10%T CGCGTGACAATGCTGGTAGAC3'
G80%G90%

B6   5'CGCCGACACCGCGGTCTACTACTGTGCGCGC T1/2 TT GC25%C TATTGGGGTCAGGGCT3'
                                       C    CC  A75%
                                       A    AG
                                       G    GB

L2   5'GTGACCATCACCTGT CAA T30%CG AGT C75%AA T30%T10%C C10%T40%T90%
                       AG  G70%       A10%   C10%C30%  A50%C10%A10%
                                      G15%   A30%A60%  G40%A10%
                                             G30%          G40%

A70%T20%T70% C5% T5% T90% T90%T10%C C70%TA20% AC40%T TGGTAACAGCAGAAGCCAGGT3'
G30%A40%A30% A90%C20%A10% C2% C5%   A20% G80% GA40%
   G40%      G5%.A40%     A6% A85%  G10%      G20%
                G35%      G2%

L3   5'AAGGCTCCAAAGCTGCTGATCTAC T14.5%TT A40%T20%T AC5% C AC20%T70% CTA
                                C28.5%CC G60%C70%   A10%   A60%A30%  G
                                A28.5%AG     A7%    G85%   G20%
                                G28.5%G      G3%

C20%C70%C20% T7C%CT GGTGTGCCAAGCCGTTTCAGCGGTAGCGGT3'
G80%A30%A80% C15%
             A15%

L5   5'ATCGCCACCTACTACTGC CT10%A CT20%T10% T60%C30%G T35%T5% T T15%T5% C
                          A90%   A80%G90% A10%G70%  C5% C20%   C10%C20%
                                          G30%      A40%A50%   A6C%A75%
                                                    G20%G25%   G15%

T14.5%TT CT15%C90% T14.5%TT ACGTTCGGCCAAGGTAC
C28.5%CC  C70%A10% C28.5%CC
A28.5%AG  A15%     A28.5%AG
G28.5%G            G28.5%G
```

Notes for Tab. 1

The random nature of the oligonucleotides of set B was limited in a manner which generates approximately the relevant amount of frequent amino acids in each position of the hupervariable regions (in accordance with the tables of Kabat et al, loc. cit.). In this strategy the number of expected stop codons was also reduced even further. In contrast with the oligonucleotides in set A, a restriction site for MluI was not introduced.

TAB. 2

CONSTRUCTION OF THE VECTOR pFMT FOR THE EXPRESSION AND SECRETION OF ANTIBODIES IN BACTERIA

DNA OF THE VARIABLE DOMAIN OF A HUMAN LYSOZYME ANTIBODY

⇓

INTRODUCTION OF RESTRICTION SITES BY SITE DIRECTED MUTAGENESIS

⇓

SYNTHESIS OF THE LEADER SEQUENCE OF PECTATE LYASE AND OF THE RIBOSOME BINDING SITE

⇓

LIGATION INTO BACTERIAL EXPRESSION PLASMIDS

⇓

P/O	RBS	P1	VH	RBS	P2	VL

P/O: promoter/operator, RBS: ribosome binding site, P2: leader sequence of pectate lyase, VH: variable domain of the heavy chain, VL: variable domain of the light chain

TAB. 3

Sequences of the leader sequences P1 and P2 in the antibody operon, and of the Tag sequences

P1

Leader sequence of pectate lyase (P1)

```
 M  K  Y  L  L  P  T  A  A  A  G  L  L  L  L  A  A  Q  P  A  M  A  Q  V  Q  L  Q
CATGAAATACCTCTTGCCTACGGCAGCCGCTGGCTTGCTGCTGCTGGCAGCTCAGCCGGCGATGGCGCAAGTTCAGCTGCA(G)
                                                                              PstI
```

P2

```
                          RBS                        Leader sequence of pectate lyase (P2)

                                                      M  K  Y  L  L  P  T  A  A  A
(C)TGCAGCCAAGCTTGAATTCATTAAAGAGGAGAAATTAACTCCATGAAGTACTTACTGCCGACCGCTGCGGCG
  PstI    HindIII

 G  L  L  L  L  A  A  Q  P  A  M  A  D  I
GGTCTCCTGCTGTTGGCGGCTCAGCCGGCTATGGCTGATATCGGATCCAGCT
                                    EcoRV  BamHI
```

The nucleotides in parentheses are the adjacent nucleotides of the plasmid

The leader sequences were synthesized by hybridization of the following oligonucleotides.

P1

a. 5'CATGAAATACCTCTTGCCTACGGCAGCCGCTGGCTTG3'

b. 5'TTAACTCCATGAAGTACTTACTGCCGACCGCTGCG3'

c. 3'ACGTCGGTTCGAACTTAAGTTTTAACTCCTCTTTAATTGAGGTACTTCATGAATGACGGCTGGCGACGCCGCCCAGAGGA

CGACAACCGCCGAGTCGGCCGATACCGACTATAGCCTAGGTCGA5'

d. 5'GCTCAGCCGGCTATGGCTGATATCGGATCC3'

e. 5'GCGGGTCTCCTGCTGTTGGCG3'

The Tag sequences were synthesized by hybridization of the following sequences:

a. 5'CCTTAGTCACAGTATCCTCAGAAGGTGAAGAATTCTA3'

b. 5'AGCTTAGAATTCTTCACCTTCTGAGGATACTGTGACTAAGGAGCC3'

TAB. 4

Nucleotide sequences of antibody DNA

a) Heavy chain (variable domain), HuVhlys HindIII.......

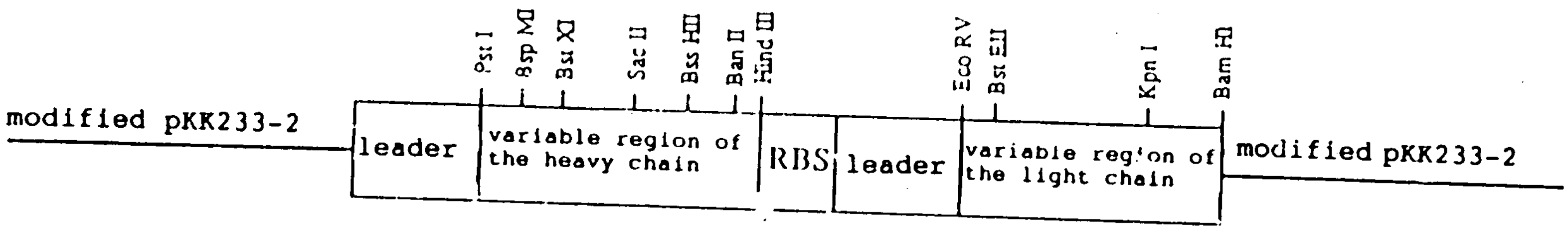
```
                        1                             10
.........G  V  H  S  Q  V  Q  L   Q  E  S  G  P  G  L  V  R
CTCTCCACAGGTGTCCACTCCCAGGTCCAACTGCAGGAGAGCGGTCCAGGTCTTGTGAGA
                                  PstI
                    20                           30     CDR1
 P  S  Q  T  L  S  L  T  C  T  V  S  G  F  T  F  S /G//Y//G/
CCTAGCCAGACCCTGAGCCTGACCTGCACCGTGTCTGGCTTCACCTTCAGCGGCTATGGT
                     BspMI
                                                 50
/V /N /W  V  R  Q  P  P  G  R  G  L  E  W  I  G /M/ I/ W/ G/
GTAAACTGGGTGAGACAGCCACCTGGACGAGGTCTTGAGTGGATTGGAATGATTTGGGGT

   CDR2             60                           70
/D /G /N /T /D /Y /N /S /A /L /K /S  R  V  T  M  L  V  D  T
GATGGAAACACAGACTATAATTCAGCTCTCAAATCCAGAGTGACAATGCTGGTAGACACC

                    80             -             90
 S  K  N  Q  F  S  L  R  L  S  S  V  T  A  A  D  T  A  V  Y
AGCAAGAACCAGTTCAGCCTGAGACTCAGCAGCGTGACAGCCGCCGACACCGCGGTCTAT
                                                 SacII
                          100 CDR3                 110
 Y  C  A  R  E /R /D /Y /R /L /D /Y  W  G  Q  G  S  L  V  T
TATTGTGCAAGAGAGAGAGATTATAGGCTTGACTACTGGGGTCAGGGCTCCCTCGTCACA
                                            BanII
 V  S  S Stop
GTCTCCTCATAAGCTTCCTTACAACCTCTCTCTTCTATTCAGCTTAA.......BamHI
          HindIII
```

b) Light chain (variable domain), Hu Vllys HindIII.....

```
                        1                            10
          G  V  H  S  D  I  Q  M  T  Q  S  P  S  S  L  S  A
CTCTCCACAGGTGTCCACTCCGATATCCAGATGACCCAGAGCCCAAGCAGCCTGAGCGCC
                     EcoRV
                    20                     CDR1     30
 S  V  G  D  R  V  T  I  T  C  R/ A/ S/ G/ N/ I/ H/ N/ Y/ L
AGCGTGGGTGACAGGGTGACCATCACCTGTAGAGCCAGCGGTAACATCCACAACTACCTG
                BstEII
                    40                           50    CDR2
/A/ W  Y  Q  Q  K  P  G  K  A  P  K  L  L  I  Y /Y/ T/ T/ T
GCTTGGTACCAGCAGAAGCCAGGTAAGGCTCCAAAGCTGCTGATCTACTACACCACCACC

                    60                           70
/L/ A/ D  G  V  P  S  R  F  S  G  S  G  S  G  T  D  F  T  F
CTGGCTGACGGTGTGCCAAGCAGATTCAGCGGTAGCGGTAGCGGTACCGACTTCACCTTC

                    80                           90    CDR3
 T  I  S  S  L  Q  P  E  D  I  A  T  Y  Y  C /Q /H /F /W /S
ACCATCAGCAGCCTCCAGCCAGAGGACATCGCCACCTACTACTGCCAGCACTTCTGGAGC

                    100
/T /P /R /T  F  G  Q  G  T  K  V  E  I  K  R..E STOP
ACCCCAAGGACGTTCGGCCAAGGTACCAAGGTGGAAATCAAACGTGAGTAGAATTTAAAC
                      KpnI

TTTGCTTCCTCAGTTGGATCC
               BamHI
```

TAB. 5

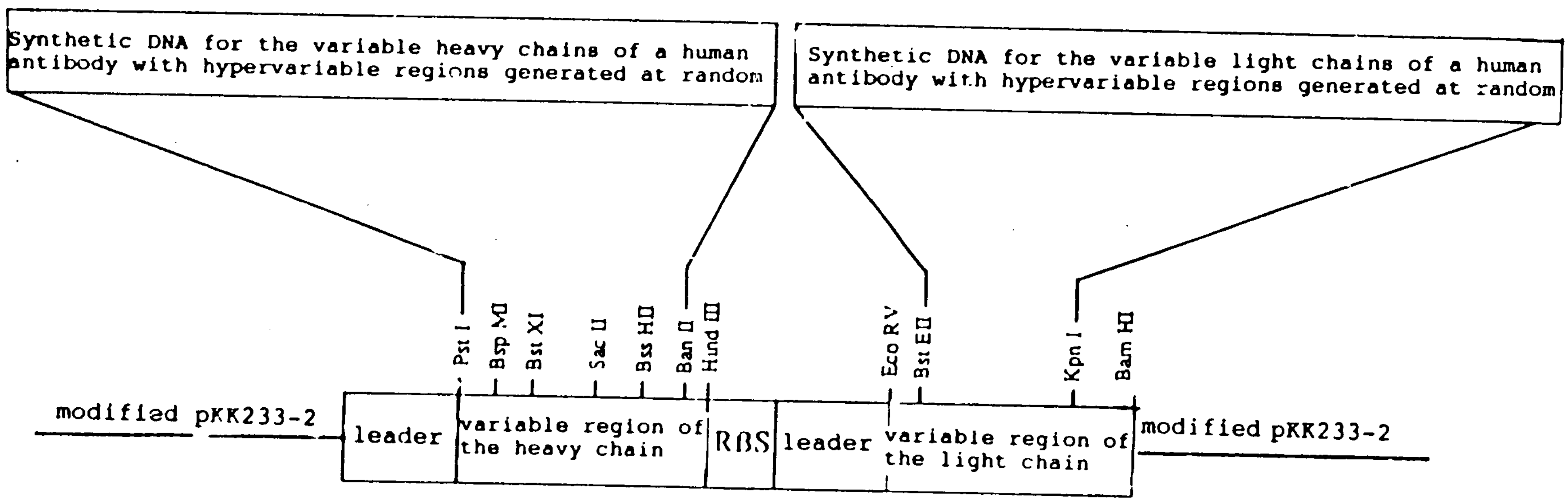
The Antibody Expression Plasmid pFMT



There is an RBS in the plasmid upstream of the heavy chain part but is not drawn in here.

TAB. 6

Insertion of the antibody libraries in the expression vector pFMT



THE EMBODIMENTS OF THE INVENTION IN WHICH AN EXCLUSIVE PROPERTY OR PRIVILEGE IS CLAIMED ARE DEFINED AS FOLLOWS:

1. A synthetic human antibody library obtainable by generating random sequences for the hypervariable regions with the limitations (a) to (e), set A or set B according to Tab. 1, and subsequent incorporation in an expression vector.

2. A synthetic human antibody library as claimed in claim 1, wherein the generated random sequences for the hypervariable regions are amplified before the incorporation in an expression vector.

3. A synthetic human antibody library as claimed in claim 1 or 2, wherein the modified vectors M13mp18HuVhlys and M13mp18HuVllys are used.

4. A synthetic antibody library as claimed in claim 1 to 3, wherein the vector pFMT is used as expression vector.

5. A process for preparing a synthetic human antibody library, which comprises generating random sequences for the hypervariable regions with the limitations (a) to (e), set A or set B according to Tab. 1, and then incorporating them in an expression vector.

6. The process as claimed in claim 5, wherein the generated random sequences are amplified before the incorporation in an expression vector.

7. The process as claimed in claim 5 or 6, wherein the vector pFMT is used as expression vector.

8. A process for isolating clones which secrete specific human antibodies, which comprises screening synthetic human antibody libraries as claimed in claim 1 to 4 using specific antigens.

9. The process as claimed in claim 8, wherein a marker

peptide, preferably the TAG sequence, is additionally incorporated and the desired clones are identified using antibodies against the marker peptide, preferably using the antibody YL 1/2.

10. The use of a synthetic human antibody library as claimed in claim 1, 2 or 3 for isolating clones which secrete specific antibodies.

11. A synthetic human antibody library as claimed in claim 1 and substantially as described herein.